INFLUENZA OLGONUCLEOTIDE LABELED WITH Cd QDs SEPARATED BY MAGNETIC PARTICLES

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INTRODUCTION

Influenza is an infectious disease caused by RNA viruses of the family Orthomyxoviridae. It spreads by droplet infection in seasonal epidemics. These epidemics occur regularly during the winter months with a six-month gap between the northern and southern hemispheres. The most important measure against infection is prevention in the form of vaccination. Influenza vaccine exists, but does not protect for whole life, is only effective for one year. This is due to mutational changes in the structure of the virus changes so that the reuse of the same vaccine the following year, this would be against a mutated virus did not protect[1]. Despite preventivation, the flu epidemic require (result in) 250 000 to 500 000 deaths annually. The greatest influenza pandemic was called Spanish flu, which took place in late 1918 and 1919, which is described as the worst pandemic (20 to 50 million victim) in human history [2]. The aim of this study was isolation and detection of CdS quantum dots labeling influezna oligonucleotide-SH (ODN-SH) H5N1. Izolation of CDs labeled influenza oligonucleotide (ODN-SH-Cd) performed with the using of nanoparticles and dual hybridization. Paramagnetic (MPs) or superparamagnetic particles are able to respond to external magnetic field, which is used for efficient separation of analytes from liquid samples. The advantage of magnetic separation is the possibility of modifying the surface of MPs, and thus the elimination of unwanted interfering adsorption of biomolecules [3]. Isolation of biomolecules using paramagnetic particles followed by electrochemical detection, is a way of detection, which is less time-consuming and laboratory equipment and is highly sensitive to even small quantities of sample.[4, 5]. For the detection of isolated Cd labeling oligonucleotides was chose electrochemical analysis. Differential pulse voltammetry was use for detection of cadmium (Cd peak), squre wave voltammetry was use for detection of oligonucleotide (peak CA).

Electrochemical methods are generally characterized by dependence on the current flowing through the electrode potential - polarization curve. Differential pulse voltammetry is one of the most widely used elektroanalytical method. In this method, the potential grows lineary inline with time and is interspersed with a voltage pulse amplitude from 10 to 100 mV. The voltage pulse is applied at the end of life mercury drops. Records the difference currents measured before entering the pulse and at the end. In contrast, square-wave voltage pulse voltammetry using positively and negatively oriented towards Potential Directive. SWV allows unlike DPV work in the high rate of change of potential and is particularly suitable for sensitive detection of reversible electrochemical systems [4].

MATERIALS AND METHODS

Izolation of Cd labeling influenza oligonucleotide (ODN-SH-Cd)

All chemicals and oligonucleotides were purchased from Sigma-Aldrich and used without further purification. For isolation of oligonucleotide was use automatic pipetting station EP motion 5075(PCR 96, Eppendorf, Germany).

In each well in the plate (PCR 96, Eppendorf, Germany) was dispensing 10 µl of Dynabeads dT_{25} (Invitrogen, Oslo), plate was subsequently transfer to the magnet and stored solution from nanoparticles was aspirate to waste, beads were further washed 3 times by 20 µl of phosphate buffer I (M, pH). The next step was first hybridization. In each wells was added 10 µl of poly A labeling anti sense H5N1oligonucleotide and 10 µl of hybridization buffer (0,1 M phosphate buffer, 0,6M guanidin thiokynate, 0,15M Tris) and the plate was incubated (15 min, 25 ° C, pipetting), folowed by washing by 20 µl phosphate buffer I (M, pH).The next step was second hybridization. . In each wells was added 10 µl of Cd labeling H5N1 oligonucleotide and 10 µl of hybridization buffer (0,1 M phosphate buffer, 0,6M guanidin thiokynate, 0,15M Tris) and the plate was incubated (15 min, 25 ° C, pipetting), folowed by washing by 20 µl phosphate buffer I (M, pH).Than was dispensing 30 µl of elution solution. Again followed by incubation (5 min, 85 ° C, pipetting). After elution was plate transfer to the magnet, and product from each well was transfer to separate well.Izolation of H5N1 oligonucleotide was followed by electrochemical detection.

Detection of Cd labeling influenza oligonucleotide (ODN-SH-Cd)

For the detection of Cd labeling influenza oligonucleotides (ODN-SH-Cd) was select electrochemical analysis. For electrochemical detection was use two voltametric methods. Differential pulse voltammetry was use for detection of cadmium (Cd peak), for detection of oligonucleotide (CA peak) was select squre wave voltammetry. Both methods were optimalized. Limit of detection for Cd and CA peak was estabilished.

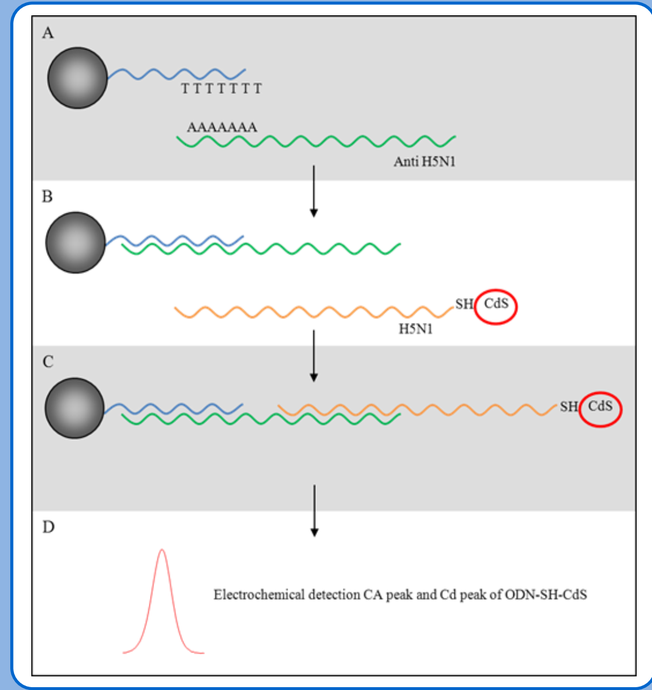

Fig.1: Scheme of H5N1(ODN-SH-CdS) electrochemical detection. A – AntiH5N1binding to magnetic particle, B – addition of ODN-SH-Cd, C – binding of ODN-SH-Cd to magnetic particle with AntiH5N1, D – electrochemical detection of ODN and Cd.

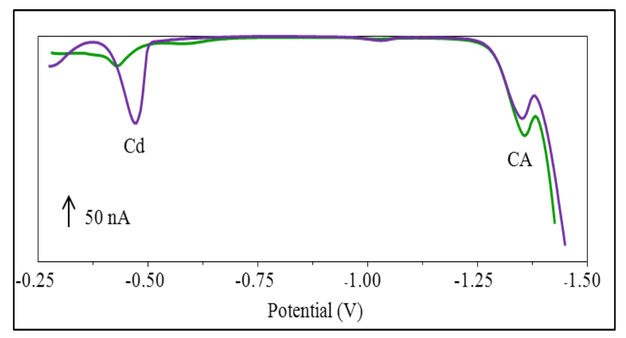

Fig.3:Comparation of voltammograms of ODN-SH (-) and ODN-SH-Cd (-).

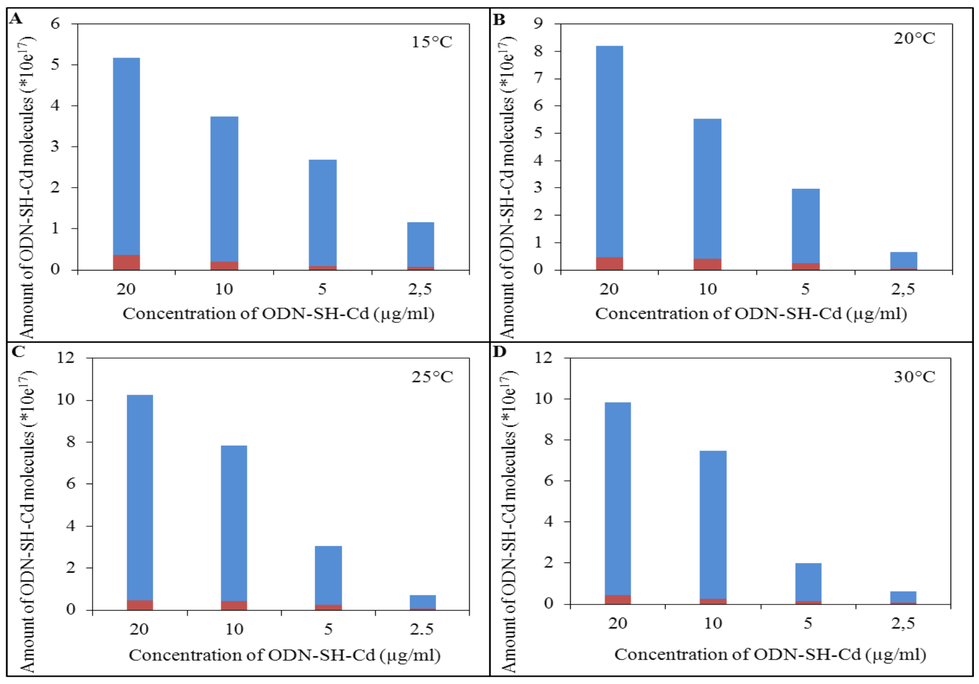

Fig.2:Amount of ODN-SH-Cd molecules (*10e^{17}) as a function of concentration of ODN-SH-Cd (µg/ml) and influence of temperatue of second hybridization **A** (15°C); **B** (20°C); **C** (25°C); **D** (30°C); referenced for Cd peak (■); referenced for CA peak (■).

Method for detection of CA peak (Cd-SH-ODN)

For detection of CA peak we used square wave voltammetry (adsorbtive transmission technology). Measurements were carried out in an electrochemical cell in the classic three-electrode system. All measurements were performed in acetate buffer (pH 5.0). Samples were deoxygenated by argon (99.99%, 120s).

Equipment: 663 VA Stand, 800 Dosino, 846 Dosing Interface. Autolab. To evaluate the results the GPES software was used. *Parameters*: initial potential 0 V; end potential - 1.85 V; frequency 10 Hz; potential step 0.005 V; amplitude 0.025 V.

Method for detection of Cd peak (Cd-SH-ODN)

For electrochemical detection of Cd peak we used the differential pulse voltammetry. All measurements were performed in acetate buffer (pH 5.0),temperature 25°C. Samples were deoxygenated by argon (99.99%, 120s).

Equipment: 663 VA Stand, 800 Dosino, 846 Dosing Interface. Autolab. To evaluate the results the GPES software was used. *Parameters*: initial potential -0.9 V; end potential - 0.45 V; deposition potential -0.9 V; duration 240 s; equilibration time 5 s; modulation time 0.06; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025

RESULTS

In this study, we described the design of conjugated MPs and QDs-based hybridization target molecule and their application for the detection of the avian influenza virus (H5N1). The hybridization complex contains from four items: (i)MPs modified oligo probes (oligo anti-sesnse) (ii) Cd QDs labeling of target oligonucleotide, (iii) capturing of target QDs labeled oligonucleotide derived from a influenza, and (iiii) electrochemical detection of metal from QDs marker and influenza derived oligonucleotide.

QDs labeling of target oligonucleotide in their combination with izolation by MPs are sensitive diagnostic tool for influenza virus detection. In order to examine the usage of MPs and QDs as tools in nucleic acid hybridization assays, we designed a MP and QD-based hybridization assay for the detection of H5N1 derived oligonucleotide. Figure 1 shows a schematic view of the hybridization procedure for the MP and QD-based probes with the target. It was designed and optimized method for isolation and detection of Cd labeling influenza oligonucleotide. Than the whole isolation part was automated by using automatic pipetting station. The effect of hybridization temperature (second hybridization) was observed.

The hybridization process is influenced by wide range of conditions such as temperature, time, pH and composition of hybridization buffer. We addressed the question of whether hybridization efficiency is affected by temperature. The hybridization process was repeated four times by temperature 15°C; 20°C; 25°C and 30°C. The most effective was hybridization temperature 25°C (Fig.2). Less effective was temperature 15°C. Electrochemical detection for QDs determination was chosen. For analysis of oligonucleotide labeled with Cd QDs two electrochemical methods were applied. For cadmium (Cd peak) differential pulse voltammetry was applied used and for oligonucleotide (CA peak) it was square wave voltammetry (SWV).

Electrochemical characterization ODN and ODN-SH-Cd (Fig. 3) shows considerable differences in voltamograms. ODN-SH voltamogram shows only one peak (peak CA) at the potential -1.35 V. Oligonucleotide ODN-SH-Cd shows two peaks. The first one corresponding to cadmium (Cd peak, potential -0.58 V) and the second one corresponds to the nucleic acid (CA peak, potential -1.36 V). There are also signifficant differences between CA peaks of Cd labeled and non labeled oligonucleotides. Non labeled ODN reported heigher and closer CA peak then Cd labeled ODN. Also peak position is divergent (labeld: -1.362V, non labeled: -1.348V)

The measured parameters were CA and Cd peak height. With increasing temperature increased amount of hybridized target (ODN-SH-Cd) and thus CA and Cd peak height. We have demonstrated the influence of hybridization temperature on the height of the CA and Cd peak.

CONCLUSION

It was proposed and optimized method for isolation of Cd labeling influenza oligonucleotide using automated pipetting station. The effect of hybridization temperature (second hybridization) on height of CA and Cd peak was observed. With increasing hybridization temperature increased amount of hybridyzed ODN-SH-Cd and thus CA and Cd peak height. The temperature optimum of second hybridization was 25°C.

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